

Oregon Wine Research Institute

Viticulture & Enology

Technical Newsletter



Welcome to the Fall 2014 Newsletter

This issue is packed with information. James Osborne, Enology Extension Specialist, OSU, opens the newsletter with an article about managing *Brettanomyces* and spoilage in the winery. Patty Skinkis, Viticulture Extension Specialist, OSU, provides an article on the challenge of yield estimation in vineyards. Jay Pscheidt, Extension Plant Pathologist, OSU, provides a timely article on scouting for crown gall. Vinay Pagay, Assistant Professor, OSU-SOREC, provides useful tips and tools on how to estimate water usage for grapevines, saving money and resources. Lastly, Walt Mahaffee, Research Plant Pathologist, USDA-ARS, provides an update on his research in detecting powdery mildew. Be sure to check out the practical guides and resources section, as we have some fantastic new resources, most of which are available online.

Cheers,
The OWRI Team

Influence of Wine Bacteria on *Brettanomyces* Spoilage

Dr. James Osborne, Associate Professor & Extension Enologist, OSU

During winemaking, the growth of *Brettanomyces* can cause the production of undesirable aromas and flavors including volatile phenols 4-ethylphenol (4-EP- Band-Aid™, medicinal smell) and 4-ethylguaiacol (4-EG - smoky, clove smell) (Chatonnet 1992). These compounds have a very low sensory threshold and are typically associated with “Brett taint” (additional information about *Brettanomyces* can be found [here](#)). The most effective way to prevent *Brettanomyces* spoilage is rigorous cleaning, sanitation, adequate additions of SO₂, and exercising caution when utilizing used barrels in your winery. In spite of this, *Brettanomyces* infections may still occur and require additional tools to prevent their growth and production of spoilage products. Our lab has been studying interactions between *Brettanomyces* and the lactic acid bacteria *Pediococcus* and *Oenococcus oeni*. These microorganisms are often present in the wine together, making us curious if any stimulatory or inhibitory relationships existed that could be utilized to better manage *Brettanomyces* spoilage.

Our initial work focused on interactions between *Pediococcus* and *Brettanomyces*, as *Pediococcus* can degrade the precursor compounds that *Brettanomyces* converts into volatile phenols. The precursor compounds, *p*-coumaric and ferulic acid, are naturally present in grapes and *Brettanomyces* converts them into volatile phenols in a two-step process (Figure 1).

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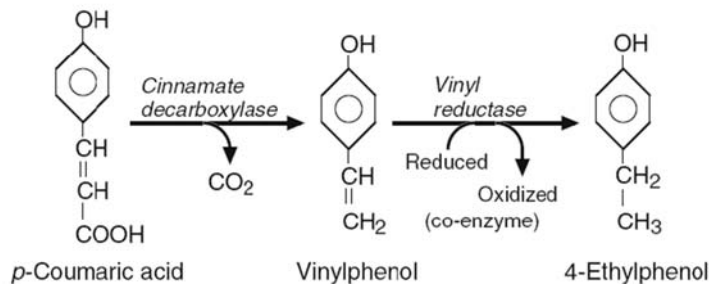


Figure 1. Pathway for 4-ethyl phenol production from *p*-coumaric acid by *Brettanomyces* (Edwards and Fugelsang 2007)

While some pediococci can perform the first step in this process, none can perform the second step that results in volatile phenols. However, our research demonstrated that if pediococci perform the first step in the process (converting *p*-coumaric acid into 4-vinylphenol) then the rate at which *Brettanomyces* produced 4-EP increased. During the course of this work, our lab, in collaboration with Dr. Charles Edwards (Professor of Enology, Washington State University) began investigating additional ways that the amount of pre-cursor compounds in wine may be affected by wine microorganisms. In particular we focused on the different forms that *p*-coumaric and ferulic acid may be present in during the winemaking process.

In grapes, *p*-coumaric and ferulic acid are typically present as esters of tartaric acid (called coutaric and fetaric acid, respectively). During winemaking, the tartaric acid may be hydrolyzed resulting in release of the free acids. This process generally occurs slowly during aging so wines can still have high amounts of tartaric acid bound *p*-coumaric and ferulic acid. This potentially represents a large pool of pre-cursor compounds for *Brettanomyces* to convert to volatile phenols. Dr. Edwards's lab surveyed a large number of *Brettanomyces* strains and concluded that *Brettanomyces* could not utilize the tartaric acid bound forms of *p*-coumaric and ferulic acid (Schopp et al. 2013). Work in our lab showed that *Pediococcus* was also unable to degrade the tartaric acid bound *p*-coumaric and ferulic acid. However, while testing commercial malolactic cultures (*Oenococcus oeni*) we discovered one strain, *O. oeni* VFO, that was capable of degrading tartaric acid bound *p*-coumaric acid resulting in the release of the free acid (Burns and Osborne 2013). This meant that Pinot noir wines where *O. oeni* strain VFO conducted malolactic

fermentation (MLF) contained significantly higher amounts of *p*-coumaric acid than wines that did not undergo MLF or underwent MLF with other *O. oeni* strains.

Are these results significant, and is volatile phenol production by *Brettanomyces* impacted? To determine this, Pinot noir wine was sterile filtered with no addition of SO₂. The wine was inoculated with either *O. oeni* strain VFO, Alpha, or VP41 and an uninoculated control was prepared. Bacterial growth and malic acid were monitored while free and tartaric acid bound *p*-coumaric acid content was assessed by HPLC. After the completion of MLF, the concentration of free or tartaric acid bound *p*-coumaric in wines inoculated with *O. oeni* strain Alpha or VP41 did not change. However, in wines inoculated with *O. oeni* strain VFO there was a decrease in tartaric acid bound *p*-coumaric and an increase in free *p*-coumaric acid. All wines were then inoculated with *Brettanomyces*, and growth and volatile phenols were monitored. *Brettanomyces* grew well in all the wines. After 40 days-growth, the wines were assessed for volatile phenol content by gas chromatography GC-MS (results can be seen in Figure 2). Because wines where MLF was conducted using *O. oeni* VFO initially contained higher *p*-coumaric acid levels, *Brettanomyces* produced significantly higher amounts of 4-EP and 4-EG. The other wines contained 4-EP and 4-EG, but at a much lower amount. In fact, informal sensory analysis of these wines revealed that the levels produced were barely detectable, while wine where *O. oeni* VFO had performed the MLF smelled distinctly like Band-Aids™ due to the high amount of 4-EP produced by *Brettanomyces*.

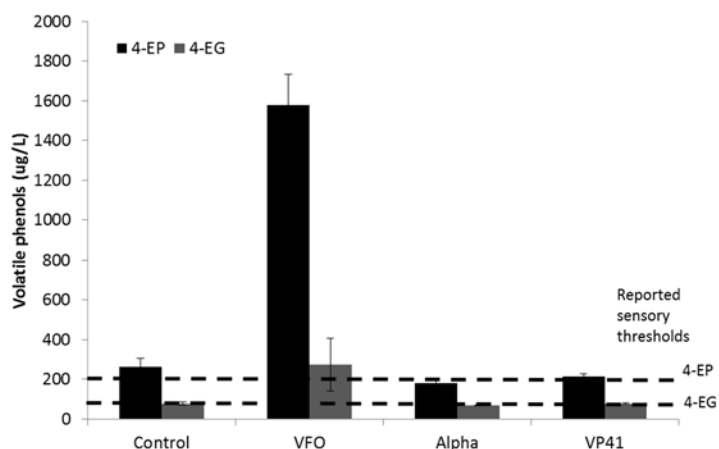


Figure 2. Production of 4-ethyl phenol (4-EP) and 4-ethyl guaiacol (4-EG) by *Brettanomyces bruxellensis* in Pinot noir wine where MLF was conducted by *O. oeni* VFO, Alpha, or VP41. (Chescheir and Osborne, unpublished).

How can a winemaker practically apply this information? It is important to understand that the conversion of tartaric acid bound *p*-coumaric acid to free *p*-coumaric acid by *O. oeni* does not itself cause wine spoilage issues. *p*-Coumaric acid is naturally found in wine and plays a positive role in the formation of stable red color compounds (Boulton 2001). Also, there may already be sufficient free *p*-Coumaric acid in your wine so that growth of *Brettanomyces* will result in high amounts of volatile phenols being produced regardless of *O. oeni*. However, having an increased amount of *p*-coumaric acid in your wine due to growth of certain *O. oeni* strains may lead to a significant increase in the amount of volatile phenols produced if a *Brettanomyces* infection occurs. So while wineries must continue to use sound winemaking practices to prevent the growth of *Brettanomyces*, using *O. oeni* strains for MLF that can degrade tartaric bound *p*-coumaric acid should be avoided in barrel-aged red wines that are most susceptible to *Brettanomyces*. In addition, care should be taken when conducting spontaneous MLF as you do not know if any of the strains conducting the MLF are capable of degrading tartaric acid bound *p*-coumaric acid. In light of this, our lab is continuing to screen commercial *O. oeni* strains for this trait. We will also be isolating *O. oeni* strains from spontaneous MLFs in order to better understand how common the ability to degrade tartaric acid bound *p*-coumaric acid is amongst *O. oeni* strains. For additional information regarding the malolactic bacteria cultures you are currently using I suggest contacting your supplier.

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The Challenge of Estimating Yields

Dr. Patty Skinkis, Associate Professor and Viticulture Extension Specialist, OSU

The 2014 season will be remembered for record-breaking dimensions. Across the state, vineyards performed beautifully with the long, warm season to ripen the higher base yields (Figure 1). Although a large crop year was apparent shortly after fruit set, many growers and winemakers were surprised at the final yields harvested. For many, actual yields deviated significantly from estimates despite careful measure of lag phase weights, counts and calculations.

Precise yield estimates are typically a trademark of a successful Oregon grape grower. Many take pride in the pain-staking procedures employed at lag phase to forecast yields each year. Grower contracts are based on yield parameters (Uzes and Skinkis, in progress) and wineries prepare for a specific capacity. When yields are off, havoc can ensue in harvest logistics and winery processing.



Figure 1. High yields in 2014 were due to high fruitfulness and fruit set, leading to increased cluster numbers per vine and cluster weight, respectively. Shown here is a Pinot noir vine from the research block at Stoller Family Estate Vineyard, Dayton, OR.

Better methods for yield estimation have become an important topic of discussion for industry and researchers alike. The industry standard is to conduct yield estimation during lag phase by counting clusters on reference vines within producing vineyard blocks and applying an increase factor to measured cluster weights. Thereafter, adjustments are made by cluster thinning vines to reach target yields. The yield estimation process must be completed during the lag period as this is when fruit

weight plateaus at approximately half the weight anticipated at harvest. Yield estimation methods are time consuming and somewhat difficult to target since lag phase encompasses a relatively short window of time. It may take growers a week or more to advance through all the blocks necessary for estimation, making it difficult to apply an appropriate increase factor. New advances in sensor technology applied to the fruiting wire have made it possible to better identify the lag period for more precise yield estimation (Blom and Tarara 2009, Tarara et al. 2013). However, until this technology is readily available in a user-friendly format, growers must consider some practical information to improve their yield estimation practices.

For years, Oregon growers have identified the timing of lag phase in Pinot noir by testing for seed hardness (50% hard seed tips when cut) or by using a standard metric (50 to 55 days post bloom) (Price, unpublished). However, there has been great debate about whether to count days from first bloom, 50% bloom, or full bloom. To improve our understanding of when lag phase occurs and how much cluster mass increases between lag and harvest, we monitored Pinot noir cluster weights since 2009. A summary of that work is outlined here for 2011 through 2014.

A 50-cluster sample was collected weekly, starting at the pea-size stage (2012-2014) or bunch closure (2011) and continued through harvest. Whole clusters were randomly selected throughout the same Pinot noir vineyard (Figure 1) on each sampling date. The vineyard was planted in 1998 to Pinot Noir 115 grafted to 101-14 rootstock. From 2012 onward, sampling increased to twice weekly just prior to or just after estimated lag phase to better determine the mass plateau. Data collection included cluster weight, rachis weight, berries/cluster, berry weight, and berry diameter. Seed hardness was quantified on 100 randomly selected berries to determine the percentage of seed hardening prior to lag phase. Percentage of berries colored was quantified on clusters until 100% berry color was reached. Total soluble solids were measured weekly from véraison to harvest.

Identifying lag phase

Lag phase defines a stage of berry development where berry mass plateaus as it transitions into ripening. By monitoring berry development each year, we retroactively 4 determined when the berry weight reached its point of

inflection. This is defined as the “actual” lag phase. Both cluster and berry weight development resulted in the classic double-sigmoidal development curve, and lag phase was identified at the inflection point (Figure 2). The occurrence of lag phase was compared across years by using the number of days post 50% bloom (DPB) and growing degree days (GDD_{50}). Results show that the number of DPB is a better indicator of lag phase than GDD_{50} , with the four year average being 51 days from 50% bloom to the start of lag phase (Figure 2, Table 1). Despite years with highly variable GDDs, the days post bloom was within the same range. Results of this work shows that the duration of lag phase in Pinot noir is short, ranging from five days in 2013 to as many as eleven days in 2012 (Figure 2, Table 1).

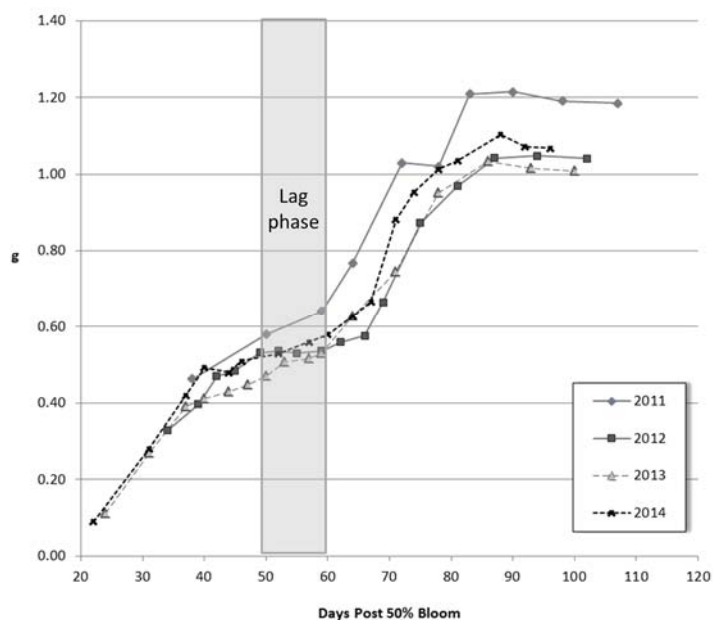


Figure 2. Increase in berry mass during development post-fruit set through harvest in 2011-2014. The general lag phase period is outlined. See **Table 1** for further details on occurrence of lag phase.

Results reported by other researchers (Sweetman et al. 2012, Tarara et al. 2013) show similar development curves with short lag periods. Since the lag phase window is so short, growers are advised to begin yield estimations first in those blocks that undergo bloom earlier and transition to those that are later. This will help ensure that data are collected within the appropriate lag window and avoid over- or under-estimating yields. Many growers have used the seed hardness test to identify lag. However, when we compared seed hardness with cluster and berry weight advancement, nearly all seeds had some level of resistance to cutting with a scalpel by the time lag was reached each

year. Therefore, seed hardening is not useful in determining the start of lag phase and may lead to incorrect estimations. Similarly, Tarara et al. (2013) found that standard practices for identifying lag led to growers identifying lag too late.

How much will cluster weight increase after lag?

An increase factor is applied to cluster weights at lag to estimate the final cluster weight at harvest. Typically, it is thought that clusters are 50% of their final weight at lag, and a simple doubling is often applied. While there are clear differences in cluster size between years, results show a relatively uniform cluster increase factor. This was an interesting finding since growers often claim that small clusters at lag will not double in size while larger clusters will easily double. Across all four years, the increase factors ranged from 1.8 to 2.0, with heavy yielding years (2011 and 2014) having similar increase factors as lower yielding years (2012 and 2013) (Table 1).

Table 1. Increase factor (IF) for cluster mass using industry standard days post bloom and actual lag period

Year	IF (50 to 55 DPB to harvest)	IF (actual lag to harvest)	Lag occurrence (DPB)	Cluster mass (g) at lag
2011	1.9	1.8	50 - 58	90.3
2012	1.9	1.9	49 - 59	57.8
2013	1.9	1.8	53 - 57	50.6
2014	2.1	2.0	52 - 60	88.9

DPB - Days post 50% bloom

The discrepancy in our data and that of growers' experience may be due to identification of lag later than the physiological lag phase in years with small clusters. Based on the data we have to date, it appears that the cluster size begins to level off by 85 DPB.

What resulted in the high 2014 yields?

Years with high whole vine yields are a result of two factors: increased cluster weight and/or higher fruitfulness. Greater cluster weights are typically a result of more berries per cluster, not increases in berry size. The high yielding years of 2011 and 2014 had greater berries per cluster, and berry weight was not different statistically (Table 2). What our 2014 data does not reflect is the higher fruitfulness that was observed across Oregon and quantified through our other studies. With greater fruitfulness, there are greater numbers of clusters per vine. When estimating yield, it is critical to count cluster number per vine and not rely on historical cluster numbers. For example, if you measured cluster weights

this year but applied a historical cluster number per vine or last year's counts, your estimates may have been off by as much as 10-20% due to this factor alone.

Table 2. Pinot noir cluster and berry size at harvest 2011 - 2014

	Year	Harvest Date	Harvest DPB	Cluster mass (g)	Berry count per cluster	Berry mass (g)	Berry diameter (mm)	TSS (*Brix)
Means	2011	20-Oct	107	161.9	131	1.19	.	.
	2012	10-Oct	102	67.1	67	0.95	11.3	22.3
	2013	17-Sep	100	92.8	88	1.01	11.3	20.7
	2014	12-Sep	96	180.4	163	1.07	11.7	24.1
Standard Deviation	2011	20-Oct	-	22.9	20	0.07	.	.
	2012	10-Oct	-	31.7	31	0.16	0.7	0.1
	2013	17-Sep	-	33.1	31	0.17	0.6	0.2
	2014	12-Sep	-	47.6	45	0.08	0.9	0.1

DPB - Days post 50% bloom.

Summary

Our data suggests that the industry standard of using 50 to 55 day post-bloom is suitable for estimating the timing of lag phase, particularly for Pinot noir in the Willamette Valley. If yield estimates are carried out during lag, then a near-doubling of cluster weight is expected. However, modifications to the increase factor will be required if yield estimates span beyond the lag period which we found to range from a few days to more than one week. It is most important that sufficient cluster weight data is collected at both lag and harvest to allow for customized increase factors to be calculated in each vineyard block. This post-harvest period is a perfect time to reflect on the season and calculate your own increase factors based on lag and harvest cluster weight data you have accumulated over the years. If you have been lax on gathering cluster data at harvest, develop protocols for future years to ensure these data are collected. A little more time spent gathering and analyzing data may increase your accuracy in estimating yields in the future.

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- Tarara, J.M., B. Chaves, L.A. Sanchez, and N.K. Dokoozlian. 2013. Analytical determination of the lag phase in grapes by remote measurement of trellis tension. *HortScience*. 48: 453-461.

Estimating the Water Use of Grapevines

Dr. Vinay Pagay, Assistant Professor, OSU-SOREC

In regions dependent on freshwater irrigation, it is critical to understand the amount of water needed for optimizing plant growth, especially as freshwater becomes scarcer in arid and semi-arid regions. Grape yield and quality are strongly dependent on vine water status and irrigation. A mismatch between the amount of water applied and actual crop water use leads to inefficient water use by the plant, which may result in poor vine health and lower economic returns over time.

To effectively manage irrigation, it is important to understand crop water use and develop methods that allow sustainable vine growth and responsible use of water. This is a main focus of my research program in southern Oregon. The first step in realizing this goal is to determine the suitability of regional weather data in estimating vine water use by “ground-truthing” with data obtained from whole-canopy transpiration measurements.

Estimating vineyard and vine water use

In many irrigated crops, growers often utilize evapotranspiration (ET) to determine how much irrigation water to apply. ET is the total volume of water lost to the atmosphere through evaporation and transpiration, including surface evaporation from the vineyard floor and transpiration from grapevines, cover crops, and weeds. Growers can obtain regional ET data from an on-site weather station, or from a regional weather station that reports these values. Additional information required to calculate ET is a crop coefficient (K_c). This is a crop-specific parameter that helps reflect changes in the plant’s size and light interception, and thus water use throughout the growing season. The K_c is different for each crop and is dependent on the stage of growth (canopy size), crop architecture, transpiration surface (predominantly exposed leaf area), and resistance to transpiration due to stomatal regulation of water.

The crop coefficient can be determined from the following ratio:

$$K_c = ET_c / ET_{ref}$$

ET_{ref} is the reference evapotranspiration of short, green, well-watered grass or taller cover crop such as alfalfa. It is the value reported by regional weather stations.

To determine site-specific crop coefficients, growers can use a ‘Paso Panel.’ (Figure 1). The [Paso Panel](http://cesanluisobispo.ucanr.edu/Viticulture/Paso_Panel/) is used to measure the percent shaded area and consists of a rectangular grid of solar cells (available as a rollable solar cell sheet) mounted on a rigid frame; the solar cells are connected to an ammeter. Measurements are taken at solar noon, when the sun is directly overhead, which is between 1:00-2:00 PM in Oregon. Readings are taken directly below the canopy but must be used when sunlight is sufficient to allow maximum current. The crop coefficient (K_c) is calculated by using the output from the panel, row spacing, and length of panel, and multiplying by a constant. For more information, please see the following website: http://cesanluisobispo.ucanr.edu/Viticulture/Paso_Panel/. The Paso Panel was used in our southern



Figure 1. A Paso Panel can be constructed to measure the canopy shaded area for calculating the crop coefficient (K_c) for grapevines in the field. Measurements are taken at solar noon when the smallest shadow is cast by the canopy (not the case in this photo). (Photo by Vinay Pagay)

Oregon irrigation research projects during 2014. We determined that K_c was 0.59 for Tempranillo and 0.66 for Grenache grapevines with a full canopy at mid-season and post-véraison. Vines with significant canopy management or under deficit irrigation are expected to have lower K_c values due to smaller or less dense canopies.

Once the ET_{ref} and K_c values are obtained, then crop evapotranspiration (ET_c) can be calculated as follows:

$$ET_c = ET_{ref} \times K_c$$

Whole canopy measurements of vine water use

Vine water use can be estimated using single leaf or whole canopy measurements of transpiration. Researchers

commonly use single leaf measures, as instrumentation is more typically available to collect these data. Canopy-scale measurements are much more difficult, as they require enclosing the entire grapevine canopy when taking measurements. We were able to measure whole vine water use in a research trial in southern Oregon by installing custom-built Mylar™ chambers (balloons) over vertically shoot positioned single-curtain Tempranillo vines (Figure 2). The vines were 14 years old, spaced at 10' x 8', and received full-irrigation (100% of ET_c) for the entire season once the midday stem water potential reached -1.0 MPa. An infra-red gas analyzer was connected to the balloons to determine whole vine transpiration. Measures of transpiration were taken approximately three weeks post-veraison and over a 24 hour period, starting on August 28, 2014. We were able to determine whole vine water use (transpiration; E_{canopy}), and water use efficiency (WUE_{canopy}) from a single whole canopy chamber (Figure 3). The E_{canopy} reached its maximum value of (0.6 gallons/vine/hour) near 6:00 PM when the vapor pressure deficit was the highest (Figure 3a, c). Vapor pressure deficit (VPD) is the difference between the amount of moisture in the air and how much moisture the air can hold. The transpiration rate we observed was consistent with rates measured in other studies using single curtain vines (Dragoni et al. 2006). Vine water use on a daily basis post-veraison was approximately 8.2 gallons.

For comparison, we calculated the daily vine evapotranspiration (ET_c) using an ET_{ref} value obtained from an on-site weather station and our measured K_c value of 0.59 for the specific canopy. The ET_c was approximately 6.3 gallons/vine/day, which was slightly lower than the whole vine transpiration that we measured of 8.2 gallons/vine/day. This 1.8 gallon difference represents the transpiration volume not accounted for by ET_c calculations and may be attributed to night transpiration or water loss (E_{night}). Based on our balloon data, E_{night} was nearly 2.6 gallons/vine/day, representing approximately 32% of the total daily water use of the vine.

Diurnal vine water use efficiency

Vine water use efficiency is defined as the ratio of net carbon assimilated by the plant to the amount of water transpired. It is a key target for improvement in grapevine production, particularly in semi-arid and arid climates where water scarcities continue to increase. In the study

where we monitored well irrigated grapevines, whole vine water use efficiency (WUE_{canopy}) decreased during the day as transpiration continued to increase (Figure 3d). This trend was maintained for most of the evening and through part of the night when it became negative as net carbon fixed by the vine was negative (due to dark respiration).



Figure 2. Whole-canopy chambers (“balloons”) used in a research block to measure the water use of fully-irrigated Tempranillo grapevines at Abacela Winery, Roseburg, Oregon. (Photo by Vinay Pagay)

The highest WUE_{canopy} values were observed early in the day, around 8:00 AM, under conditions of moderate sunlight and VPD, possibly due to low transpiration at that time. This finding is consistent with other published studies on well-watered vines that reported the highest WUE_{canopy} occurs early in the day (Poni et al. 2014). Interestingly, in our study, the highest net carbon assimilation rates were observed at 2:00 PM (data not shown) and did not coincide with the time when the highest WUE_{canopy} was observed at 8:00 AM (Figure 3d). The lower WUE_{canopy} values observed during the mid-afternoon were likely due to higher transpiration (E_{canopy}) due to high VPD conditions of the well-watered vines; (Figure 3c) and lower net carbon assimilation rates (due to higher canopy temperature, which limits photosynthesis and increases respiration).

Summary

Growers may use a number of approaches to determine vine water use. In this article, we compared the practical approach of estimating ET_c with regional weather station data to whole-canopy transpiration, a method that is

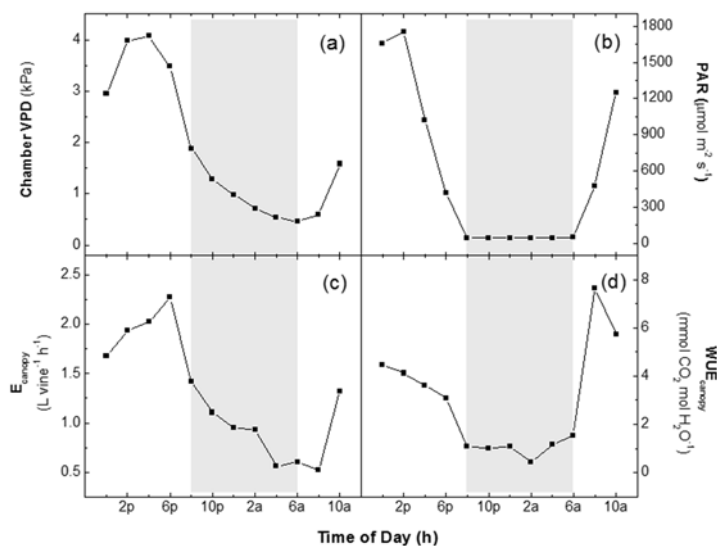


Figure 3. (a) Vapor Pressure Deficit inside the balloon chamber; (b) Photosynthetically Active Radiation (400-700 nm) inside the chamber; (c) Whole-canopy transpiration or vine water use (E_{canopy}); (d) Whole-canopy water use efficiency (WUE_{canopy}) calculated as the ratio of canopy net carbon exchange rate (NCER; data not shown) and canopy transpiration. Measurements were made over 24 hours, starting at 12:00 PM on August 28, 2014, three weeks post-véraison. Areas shaded in grey indicate night.

feasible with appropriate research instrumentation. Our measurements of E_{canopy} indicate that mature, well-watered grapevines with a full-canopy of leaves can use over 8 gallons of water per day under warm, dry conditions. Comparison of E_{canopy} with ET_c revealed that ET_c may be underestimating the actual water needs of the vine since it does not account for night water loss (E_{night}), which was found in this study to represent approximately one-third of the total daily water use of the vine and was responsible for lowering daily vine water use efficiency. This important finding has implications for irrigation scheduling of grapevines, particularly in southern Oregon, eastern Oregon, California, and Washington. In using the ET approach, growers can compensate for night water losses during warm periods by either increasing their weekly irrigation applications, or maintaining smaller canopies. In all cases, it is recommended that growers monitor vine water status and/or stomatal conductance regularly throughout the growing season using instruments such as the leaf pressure chamber and leaf porometer, respectively. These tools will aid in irrigation scheduling – both amount and timing – to ensure that vines do not experience levels of water stress that are detrimental to yield and fruit quality.

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Further Reading

The Evapotranspiration Method for Irrigation Scheduling. eXtension.

<http://www.extension.org/pages/32939/the-evapotranspiration-method-for-irrigation-scheduling#.VE6CsXfyfAk>

Using the Paso Panel to quickly measure the canopy shaded area and estimate vineyard irrigation crop coefficients. UCCE San Luis Obispo County.

http://cesanluisobispo.ucanr.edu/Viticulture/Paso_Panel/

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Time to Look for Crown Gall

Dr. Jay W. Pscheidt, Professor and Extension Plant Pathologist, OSU

The dormant season is a time to scout for crown gall, a disease caused by a little bacterial genetic engineer. Crown gall is most commonly found in regions with cold winters such as southern and eastern Oregon. Many regions of the Pacific Northwest experienced unusually cold temperatures during the 2013-2014 dormant season. As a result, vines may have developed crown gall this past growing season. This article provides information on crown gall symptoms, causes, and both cultural and chemical management tactics.

Symptoms

Fleshy, hard galls are typically found on the trunk, located at the root crown and on the first two feet of the vine above the soil line (Figure 1).



Figure 1. Symptoms of crown gall are shown along the trunks of this vine. Having vines trained with double trunks, as shown above, can be helpful in renewing a galled vine if only one trunk is damaged. Luckily, these are own-rooted vines, and the sucker visible in the background can be used to retrain the vine.

Large galls may develop rapidly, blocking the vascular system and completely girdling young vines in one season. Galled vines generally produce poor shoot growth, and

vine portions above the galls may wilt, become chlorotic, and die due to the gall blockage. Few clusters develop on these shoots, and if fruit does form, it often shrivels or will not ripen. Grape yields can drop by 40% on affected vines. Crown gall is a greater problem on grafted vines than own-rooted vines, as the graft union can result in active gall growth even without winter freezes or mechanical damage. Normal callus growth at the graft union or at the end of cane cuttings used for propagation can be mistaken for crown gall; however, these will form normal vascular connections while crown gall will not. Testing of plant materials by an appropriate laboratory can identify the difference during early callus growth.

Cause

Crown gall is caused by *Agrobacterium vitis* (which may also be called *Rhizobium vitis*), a bacterium that can attack grapevines. The bacteria can survive for years in old galls, infected vines, and infected plant debris in the soil. It can enter the plant through wounds, such as those made by grafting, mechanical damage, cold damage, or pruning cuts. More often than not, the bacterium has already infected the vascular tissues of symptomless grapevines. This bacteria is a genetic engineer that modifies the grapevine to make it a home and produce a food source. Winter damage that causes minor wounds can trigger the infection process. After the bacterium enters a wound, a small piece of its DNA is transferred into the plant's DNA. The foreign DNA transforms normal plant cells in the wounded area into tumor cells. Once transformed, tumor cells proliferate automatically and produce unique substances that the bacteria can readily use as a food source. The result is a gall of disorganized hyperplastic and hypertrophic tissue.

An important point to remember is that the bacteria do not need to be alive in the galled tissue for crown gall to continue to be a problem. The main issue is that a bit of bacterial DNA is now part of those plant cells, which continue to grow until cut off from the vascular system.

Cultural Management

Cultural management tactics to reduce or eliminate crown gall include planting crown gall-free vines, preventing vine injury, and removing diseased tissue or vines.

Growers can purchase crown gall-free vines from nurseries thanks to the efforts of the *Clean Plant Center Northwest*

(CPN) - Grape Program in Washington. The CPN produces vines free of crown gall bacteria and other plant pathogens, making certified vines available to nurseries to propagate. Nurseries produce crown gall-free vines by propagating from green shoot tips which will not harbor the bacteria. They can also soak dormant cuttings for 30 minutes in hot water (129°F or 54°C) to help eliminate most of the bacteria. This hot water soak will kill primary buds, but secondary buds should grow well. Resistant rootstocks are available, but grafting will not allow for retraining of vines after severe winter cold damage.

To avoid cold damage to grapevines, it is important to choose a vineyard location where rapid temperature changes in the fall and spring are not common. Control of soil borne nematodes prior to planting may help reduce the incidence of plants with galls. If you have a vineyard that has frost or freeze pockets, wind machines can be used to raise surface air temperature during temperature inversions. Also, avoid any injury near the base of the vine, which will help to limit this and other trunk diseases. If you find crown gall, remove diseased vines including as much old root material as possible from the vineyard. Sterilize pruning tools between vines with a disinfectant such as bleach or shellac thinner. If the gall is high enough on the trunk, a sucker replacement may be brought up from well below the galled areas in non-grafted plants. In areas with repeated winter injury and crown gall development, use of a multiple trunk training system may allow for trunk replacement without losing an entire vine. Also, do not graft over to a new cultivar on vines that are exhibiting crown gall.

Chemical control

Efficacy of chemicals such as copper-based compounds, other chemicals and antibiotics is not satisfactory and thus not recommended for control. The product Gallex® can be painted onto very young galls to reduce further gall development. It works much like a contact herbicide killing plant cells that contain the bacterial DNA. Tissue surrounding the gall may also be injured, especially on younger plants. Galls may return the next year, or if treated late, galls may continue to develop. Gallex® is not generally recommended for use because it is often too labor- and time-intensive to apply.

Biological control

Agrobacterium radiobacter K84 (Galltrol®) is ineffective for grapes since it does not prevent infections. Non-pathogenic *Rhizobium vitis* strain ARK-1 and others have been shown to be effective but are not yet commercially available.

Further Reading

For more information about crown gall or other diseases in grapevines, check out the *Pacific Northwest Disease Management Handbook* (<http://pnwhandbooks.org/plantdisease/>).

Fun Fact

The name crown gall comes from the location and type of symptoms generally produced by *Agrobacterium*. Ask a forester where the “crown” of a tree is, and they will point to the top with all its branches and leaves. However, the plant disease “crown gall” usually occurs near or below the ground level on most plants. The word “crown” is really a shortened term for the phrase “root crown” or the top of the root system. The word “gall” means a growth or swelling where extra, unorganized plant cells are produced. Crown gall is the common name of many diseases that these bacteria produce.

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- Kawaguchi, A. 2013. Biological control of Crown Gall on grapevine and root colonization by nonpathogenic *Rhizobium vitis* strain ARK-1. *Microbes and Environments*. 28:306-311.

Using Inoculum Detection in Managing Grape Powdery Mildew

Dr. Walt Mahaffee, Research Plant Pathologist, USDA-ARS

Grape powdery mildew is a perennial problem in most Oregon vineyards, and it seems to explode to economically damaging levels overnight despite your best efforts to manage it. In other years (e.g. 2014), you are often left wondering why you worked so hard trying to stay ahead of what never happened. The reasons for this are many and intertwined. On and off over the past 19 years, my research group has been exploring the nuances of grape powdery mildew development in the Pacific Northwest. This story is an example of how science is an iterative process that takes many twists and turns before sometimes stumbling into a solution.

Upon starting my job in August 1996, and after long discussions with Jay Pscheidt (Extension Plant Pathologist, OSU), Ed Hellman (former Extension Horticulturist, OSU), and numerous grape growers throughout the Willamette Valley, I began asking myself two questions: 1) Why was the grape powdery mildew epidemic so different from year to year despite similar summer environments?, and 2) What was different in the spring weather that impacted disease development?

Why these questions you might ask? Grape powdery mildew is caused by an obligate biotrophic ascomycete fungus, *Erysiphe necator*. It can only reproduce on a living host and has both sexual and asexual reproduction. The result is that the pathogen can overwinter as cleistothecia (fruiting bodies produced during sexual reproduction) on vine bark or as mycelia in the dormant buds (asexual bud perenniation). Bud perenniation is extremely rare in well managed commercial vineyards but can occur on vines used in landscapes and other unmanaged plantings. We first found evidence of bud perenniation in abandoned vines at our research farm and have subsequently found it in abandoned vineyards, backyards, and other unmanaged vines throughout Oregon.

In the spring of 1997, we started moving potted grape plants in and out of research blocks every day from bud break until mid-June to monitor disease development. This allowed us to determine which day ascospore release

occurred and how it correlated to environmental conditions. There was only the occasional ascospore release event until a period of warm weather occurred, even though there were conditions that are associated with ascospore release (days with 0.1 inch of rain and daily average of 50°F) as found in New York by researchers Pearson and Gadoury (1987). An examination of historical weather data indicated that there were numerous events (20 or more in some years) between November 1st and March 1st that should cause ascospore release. Subsequent testing demonstrated that ascospore release prior to bud break did occur.

I also noticed that some Oregon and Washington vineyards did not have any signs or symptoms of powdery mildew, despite what the literature would say was a very poor management program and highly conducive environmental conditions. These observations made me question whether some vineyards even had overwintering inoculum.

In 1999 my lab was directed to work on the introduced disease hop powdery mildew, which put our grape research on the back burner for five years. However, the synergies between the two projects soon led to a close collaboration with Gary Grove (Plant Pathologist, Washington State University). Gary had noticed a similar phenomenon in commercial vineyards in eastern Washington. Grape powdery mildew was often absent at the end of the season, despite less than optimal timing or missed fungicide applications. We were also seeing the same thing in hops.

In 2001, Gary and I started to ask why vineyards and hop yards remained disease free. My group developed methods using PCR (a method of amplifying specific DNA) to detect hop powdery mildew in hop cones, while Gary visually counted the spores captured with spore traps. Gary's graduate student, Jennifer Falacy, examined whether PCR could be used to detect grape powdery mildew in the air. After three years, she developed a qualitative PCR technique for the detection of grape powdery mildew in trap samples that could detect as few as 100 spores 100% of the time and detect ascospore release in a research vineyard.

During this time, we tested various trap designs and deployment methods, including driving them around the vineyard on an ATV. Ultimately, the best location for the trap was in the area of the vineyard that had the most severe disease the prior year or the perennial hotspot.

This made us think we could use inoculum detection to initiate fungicide applications. However, convincing vineyard managers that they should risk their crop to powdery mildew by not spraying early was rather difficult. To convince them, we placed traps at various locations in and around commercial vineyards and scouted for disease on a weekly basis and asked them to examine the data each week. After two years, a few brave souls were willing to leave several rows unsprayed until inoculum was detected. Over the next five years we continued to expand the locations, plot size, and number of vineyard managers collaborating with us. We had 57 test locations over seven years. The growers saved an average of 2.3 spring fungicide applications per vineyard without increasing the economic damage caused by powdery mildew by waiting for inoculum detection before making the first fungicide application. There were no delays of fungicide applications in some vineyards, while other vineyards delayed applications until bloom.

During these experiments, the participating managers were also experimenting with how to use this data. One organic vineyard manager delayed applications over his entire vineyard (more than 100 acres) based on the trap data and had no difference in disease compared to a neighboring non-organic vineyard. Other managers used the data in conjunction with the [Gubler/Thomas disease risk model](#) to extend or tighten spray intervals. In 2010, Coastal Viticulture Consultants began offering the inoculum detection service commercially in Napa Valley with similar results in improving disease management while reducing fungicide use. However, some vineyards saw an increase in fungicide use but also saw improved disease management from previous years.

At this time, inoculum detection seemed to be an effective management tool, but it was difficult to implement commercially due the technical expertise and equipment required for the PCR procedures. Luckily, a new DNA amplification technique (LAMP-loop mediated isothermal amplification) showed promise in the medical community.

A LAMP kit was cheap, did not require refrigeration, expensive equipment, or technical skill to conduct. In the late summer of 2009, we began to develop a LAMP protocol and cheap DNA extraction technique for grape powdery mildew, and we were conducting field testing by March 2010.

We tested this approach by having vineyard managers conduct their own trapping, DNA extraction, and LAMP reactions, then compared it to results we obtained from traps placed adjacent to grower traps. We also gave them blind reference samples of 0, 1, 10, 100, or 1,000 conidia of *E. necator*. The managers were more than 50% accurate in detecting 1 or 10 spores and 100% accurate in detecting 100 or more spores. We could detect 1 spore 100% of the time. Growers had 70% agreement in detecting *E. necator* compared to our LAMP results, and our LAMP results were 89% in agreement with our quantitative PCR results. These gave us confidence that the technique could also work for growers. However, there was still room for improvement since it was somewhat difficult for growers to accurately assess if a white precipitant had formed. To make the grower assay more easily interpreted, Lindsey Thiessen, a Ph.D. student in my lab, partnered with Diagenetix, Inc. to develop a hand-held device that uses fluorescence to indicate whether spores were present. This research is showing promise but now needs a local commercial entity to finish implementation at a commercial scale.

Next steps...

In 2008, we began collaborating with engineers, including Rob Stoll (University of Utah) specializing in fluidic modeling, to understand how much of the vineyard can be represented by an individual spore trap. We have significantly improved understanding of air turbulence in vineyards and how turbulence may impact disease and pest movement and development. Our models predict the 3D shape of a spore plume and explain why we see disease moving along rows and jumping around a vineyard as opposed to radiating out from a focal point. These results led us to take the next step toward a more flexible approach to determining spore presence in vineyards that did not rely on stationary traps. We are currently working with Acutor Precision Agriculture, a local UAV (Unmanned Aerial Vehicle) company, to assess spore presence by flying over vineyards.

In 2014, the Erath Foundation donated funding to the OWRI for a terrestrial LIDAR (Light Detection and Ranging), allowing us, in collaboration with Chad Higgins (Biological & Ecological Engineering, OSU) and Chris Still (Forestry, OSU), to further explore the relationship of canopy architecture and disease development as well as light interception, photosynthetic potential, evapotranspiration, and irrigation needs of grapevines. This pathogen dispersion research could ultimately result in the development of a vineyard simulation model with expanded collaboration of other OWRI researchers.

Future vision

We envision a future where disease and pest presence and quantity is determined using molecular identification, olfactory sensing, and/or spectral imagery collected by autonomous vehicles moving above or through vineyards. These data are then integrated with modeling systems for vine growth, pathogen and pest dispersion, and disease development to generate spatially explicit risk metrics to guide precision management practices. Ultimately, these results and models will develop into a simulation model that can be used by vineyard managers in a way similar to how engineers use simulation models in their design process or how pilots learn to fly. It will be used to ask “what if” questions to better determine a course of action, to train new vineyard managers, learn how to manage a new location, or design a more efficient vineyard.

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Walt Mahaffee leads The Foliar Pathology Lab at USDA-ARS, Horticulture Crops Research Lab, Corvallis, OR. The lab currently consists of Mitchell Evers, Chris Gorman, Tara Neill, and Lindsey Thiessen. Prior members who contributed to this research are Andy Albrecht, Thomas Caye, Jim Eynard, Eric Issacs, Jessica Keune, Danielle Lightle, Andy Livesay, Amy Peetz, Cole Provence, and Bill Turechek. It took a large research team and industry collaboration to conduct this work.

Practical Guides & Resources

This section provides resources written by members of the Oregon Wine Research Institute and our partners. Many of these publications are developed and delivered through Extension and are available online, and others are from reputable trade magazines.

Dealing with Compromised Fruit in the Winery

An article written by Dr. James Osborne, OSU Enology Extension Specialist, assessing how to best handle compromised fruit in the winery. *Practical Winery and Vineyard*. August 2014. <http://www.winesandvines.com/>

Distribution and monitoring of grape mealybug: A Key Vector of Grapevine Leafroll Disease in Oregon

This guide provides a practical overview of the grape mealybug and its role as a key vector in the spread of Grapevine Leafroll Disease. This publication was released in April 2014 by *Oregon State University Extension Publishing* by authors K. Daane, C., Kaiser, R. Hilton, D.T. Dalton, V.M. Walton, and L.J. Brewer. It is available online. <https://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/47545/em9092.pdf>

Establishing a Vineyard in Oregon: A Quick-start Resource Guide

This is a great resource for anyone considering establishing a vineyard in Oregon. It is also a useful resource for new and up-to-date information sources. This publication was released in September 2014 by *Oregon State University Extension Publishing* (EM 8973) and was published by Dr. Patty Skinkis, Viticulture Extension Specialist. <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/52092/em8973.pdf>

Field Guide for Integrated Pest Management in Pacific Northwest Vineyards

The guide provides practical information about pest and disease management for grape growers and winemakers throughout the Pacific Northwest. It is beautifully illustrated and includes information about specific pests, management techniques (chemical and cultural), and IPM principles. This guide was published in June 2013 by Washington State University, Oregon State University, and University of Idaho through *Pacific Northwest Extension Publishing* (PNW 644). Edited by M.M. Moyer and S.D. O'Neal, this book is available for purchase online at <http://cru.cahe.wsu.edu/CEPublications/PNW644/PNW644.pdf>.

Improving Management of Grape Powdery Mildew with New Tools and Knowledge

Walter Mahaffee, Seth Schwebs, Francesca Hand, Doug Gubler, Brian Baily and Rob Stoll provide growers with practical tools to help fight Powdery Mildew in this article in *Practical Winery and Vineyard*. April 2014. <http://www.practicalwinery.com/>

Mobile Access to Pesticides and Labels (MAPL)

(www.npic.orst.edu/mapl): The National Pesticide Information Center at OSU developed this tool to access federal pesticide labels and information. MAPL retrieves data from two EPA databases and can be queried by product name, pest, site, and registration number. This tool functions on computers but is best displayed on mobile devices. If you want further information or have feedback on this tool, please contact Dave Stone, Associate Professor and Director, National Pesticide Information Center at OSU (Dave.Stone@oregonstate.edu, 541-737-4433).

Pacific Northwest Weed Management Handbook

This is the most comprehensive guide for weed management for the region. It is authored by Extension specialists from throughout the Pacific Northwest, and provides information on weed management strategies, herbicide lists, herbicide resistance, and more. This online handbook is edited by E. Peachey and available through *Pacific Northwest Extension Publishing*. It is updated quarterly, and the most recent revision was published in March 2014. <http://pnwhandbooks.org/weed/>

Soil Acidity in Oregon: Understanding and Using Concepts for Crop Production.

This guide provides information on how to manage soil pH for various crops. While grapes are not specifically mentioned in this publication, the concepts for testing, interpreting and managing soil pH for nutrient management are discussed. It also provides helpful information that may be used when considering cover cropping in the vineyard. This publication was released in July 2013 by *Oregon State University Extension Publishing* (EM 9061) by authors J.M. Hart, D.M. Sullivan, N.P. Anderson, A.G. Hulting, D.A. Horneck, and N.W. Christensen. It is available online. <https://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/41199/em9061.pdf>

Spotted Wing Drosophila Identification and Testing

These two documents, developed by Amy J. Dreves, Adam Cave, and Jana Lee were developed in October 2014 to assist growers in detecting and identifying Spotted Wing Drosophila larvae in fruit. These publications provide information on proper supplies, collection methods, detection parameters and more.

A Detailed Guide for Testing Fruit for the Presence of Spotted Wing Drosophila (SWD) Larvae (EM 9096)

<http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/52502/em9096.pdf>

Spotted Wing Drosophila (SWD): A quick, 7-step guide for detecting SWD larvae in fruit (EM 9097)

<http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/52501/em9097.pdf>

Vineyard Canopy Management Publication Series

A series of four articles were published by Dr. Patty Skinkis, Viticulture Extension Specialist, in collaboration with co-authors Amanda Vance and Alison Reeve (graduate research assistants) and research colleague Dr. Paul Schreiner. These publications provide information on components of canopy management including the concepts and applications of vine balance, how vine balance is altered by canopy management practices, and two protocols developed for use by industry with information about using these data for decision-making. All articles were published by *Oregon State University Extension Publishing* in June 2013 and are available online through links provided below:

Understanding Vine Balance (EM 9068) <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/39883/EM%209068.pdf>

The Role of Canopy Management in Vine Balance (EM 9071) <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/39968/EM%209071.pdf>

How to Measure Dormant Pruning Weight of Grapevines (EM 9069) <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/39902/em9069.pdf>

How to Measure Grapevine Leaf Area (EM 9070) <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/39969/EM%209070.pdf>

Research Publications

Results of research conducted in viticulture and enology are published in peer-refereed academic journals, peer-reviewed reports, or books, which validates the scientific work of the authors. The following articles were released in 2014 and describe research conducted by members of the Oregon Wine Research Institute at Oregon State University.

Viticulture

Feng H, F. Yuan, P.A. Skinkis, and M.C. Qian. 2015. Influence of cluster zone leaf removal on Pinot noir grape chemical and volatile composition. *Food Chemistry* 173:414-423. <http://www.sciencedirect.com/science/article/pii/S0308814614015374>

Gouthu S., S.T. O'Neil, Y. Di, M. Ansarolia, M. Megraw, and L.G. Deluc. 2014. A comparative study of ripening among berries of the grape cluster reveals an altered transcriptional programme and enhanced ripening rate in delayed berries. *J. Exp. Bot.* 65: 5889-5902. <http://www.ncbi.nlm.nih.gov/pubmed/25135520>

Schreiner, R. P., C.F. Scagel, and J. Lee. 2014. N, P, and K supply to Pinot noir grapevines: Impact on berry phenolics and free amino acids. *Am. J. Enol. Vitic.* 65:43-49. <http://www.ars.usda.gov/SP2UserFiles/person/5018/PDF/2013/2013%20AJEV%2064-26-38.pdf>

Schreiner, R. P., P.A. Skinkis, and A.J. Dreves. 2014. A rapid method to assess grape rust mites on leaves and observations from case studies in western Oregon vineyards. *HortTechnology* 24:38-47. <http://horttech.ashspublications.org/content/24/1/38.abstract>

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Insect, Disease, and Pest Management

Bailey, B. N., M. Overby, P. Willemsen, E.R. Pardyjak, W.F. Mahaffee, and R. Stoll. (2014). A scalable plant-resolving radiative transfer model based on optimized GPU ray tracing. *Ag. Forest Met.* 198-199;192-208. <http://www.sciencedirect.com/science/article/pii/S0168192314002032>

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Mahaffee, W.F. 2014. Use of airborne inoculum detection for disease management decisions. Pp, 39-54. *Detection and Diagnostics of Plant Pathogens*, M. L. Gullino and P. Bonants, Eds. Springer Verlag, NY. http://link.springer.com/chapter/10.1007/978-94-017-9020-8_3

Tochen, S., D.T. Dalton, N. Wiman, C. Hamm, P.W. Shearer and V.M. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental entomology*, 43: 501-510. <http://www.ncbi.nlm.nih.gov/pubmed/24612968>

Wiman, N. G., V.M. Walton, D.T. Dalton, G. Anfora, H.J. Burrack, J.C. Chiu, and C. Ioriatti. (2014). Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. *PloS One*, 9, e106909. <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0106909>

Enology

Schopp, L.M., J. Lee, J.P. Osborne, S.C. Chescheir, and C.G. Edwards. 2013. Metabolism of nonesterified and esterified hydroxycinnamic acids in red wines by *Brettanomyces bruxellensis*. *J. Agric. Food Chem.* 61: 11610-17. <http://pubs.acs.org/doi/abs/10.1021/jf403440k>